Global Change Biology (2005) **11,** 1156–1166, doi: 10.1111/j.1365-2486.2005.00967.x

Species of plants and associated arbuscular mycorrhizal fungi mediate mycorrhizal responses to CO_2 enrichment

NANCY COLLINS JOHNSON*, JULIE WOLF†, MELISSA A. REYES*, ASHLEY PANTER*, GEORGE W. KOCH* and ANDREA REDMAN*

**Departments of Environmental Sciences, Biological Sciences and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011-5694, USA, †Molecular Plant Pathology Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, MA 20705-2350, USA*

Abstract

It has been suggested that enrichment of atmospheric CO_2 should alter mycorrhizal function by simultaneously increasing nutrient-uptake benefits and decreasing net C costs for host plants. However, this hypothesis has not been sufficiently tested. We conducted three experiments to examine the impacts of CO_2 enrichment on the function of different combinations of plants and arbuscular mycorrhizal (AM) fungi grown under high and low soil nutrient availability. Across the three experiments, AM function was measured in 14 plant species, including forbs, C_3 and C_4 grasses, and plant species that are typically nonmycorrhizal. Five different AM fungal communities were used for inoculum, including mixtures of *Glomus* spp. and mixtures of Gigasporaceae (i.e. *Gigaspora* and *Scutellospora* spp.). Our results do not support the hypothesis that CO_2 enrichment should consistently increase plant growth benefits from AM fungi, but rather, we found CO_2 enrichment frequently reduced AM benefits. Furthermore, we did not find consistent evidence that enrichment of soil nutrients increases plant growth responses to CO_2 enrichment and decreases plant growth responses to AM fungi.

Our results show that the strength of AM mutualisms vary significantly among fungal and plant taxa, and that CO_2 levels further mediate AM function. In general, when CO_2 enrichment interacted with AM fungal taxa to affect host plant dry weight, it increased the beneficial effects of Gigasporaceae and reduced the benefits of *Glomus* spp. Future studies are necessary to assess the importance of temperature, irradiance, and ambient soil fertility in this response. We conclude that the affects of CO_2 enrichment on AM function varies with plant and fungal taxa, and when making predictions about mycorrhizal function, it is unwise to generalize findings based on a narrow range of plant hosts, AM fungi, and environmental conditions.

Key words: AM fungi, arbuscular mycorrhizas, carbon dioxide enrichment, CO_2, experimental scale, Gigasporaceae, *Glomus*, mutualism, mycorrhizal function, nitrogen

Received 18 August 2004; revised version received 20 December 2004 and accepted 7 January 2005

Introduction

Arbuscular mycorrhizal (AM) symbioses facilitate plant uptake of soil nutrients in most temperate and tropical ecosystems. The mutualistic effects of these associations are predicted to be sensitive to anthropogenic enrichment of atmospheric CO_2, because elevated CO_2 should simultaneously increase plants' photosynthetic rates and soil nutrient requirements (O'Neill, 1994). From a plant perspective, AM function is determined by the balance between photosynthate costs and nutrient benefits (Fitter, 1991). Allocation of photosynthate to AM fungi represents a major C cost to plants (Koch & Johnson, 1984), and increased photosynthetic rates at elevated CO_2 should make more C available to support AM symbioses (Lovelock *et al*., 1997; Jifton *et al*., 2002). Changing C acquisition costs through CO_2 enrichment is expected to increase the relative benefits of AM

Correspondence: Nancy Collins Johnson, Environmental & Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5694, USA, tel. +1 928 5236473, fax +1 928 5237423, e-mail: Nancy.Johnson@nau.edu

uptake of soil resources (Hoeksema & Bruna, 2000; Sterner & Elser, 2002).

Several studies support the hypothesis that atmospheric CO_2 enrichment will influence the balance between mycorrhizal costs and benefits. Carbon demands of AM fungi increase plant photosynthetic rates (Wright *et al*., 1998a), and C gained through AM enhancement of C assimilation is allocated to the fungus, not to increasing host plant biomass (Wright *et al*., 1998b; Miller *et al*., 2002). Furthermore, CO_2 enrichment can mitigate plant growth depressions that are generated when C costs of AM fungi outweigh their nutrient uptake benefits (Jifton *et al*., 2002). Although many studies have shown that atmospheric CO_2 enrichment increases percent root length colonization by AM fungi (Treseder, 2004), this effect generally disappears when the confounding effect of increased root biomass is factored out (Fitter *et al*., 2000). Nevertheless, elevated CO_2 often increases allocation to AM hyphae that occur in the soil outside plant roots (Rillig, 2004; Staddon *et al*., 2004).

The idea that CO_2 enrichment will decrease AM costs and/or increase their benefits for plants is clearly overly simplistic because many factors mediate AM function. Plant genotypes vary in mycorrhizal dependency (Graham & Eissenstat, 1994), AM fungal genotypes vary in mutualistic effects (Klironomos, 2003), and genotypes of associated host plants and AM fungi and environmental conditions interact to control the costs and benefits of AM symbioses (Johnson *et al*., 1997). Abiotic factors such as soil fertility (Johnson, 1993) and temperature (Gavito *et al*., 2003) can strongly impact mycorrhizal functioning. Plant taxa differ in their responses to both CO_2 enrichment and AM symbioses. Fast-growing plant species with a C_3 photosynthetic pathway have been shown to benefit more from elevated CO_2 and less from mycorrhizae than slow-growing plants with a C_4 photosynthetic pathway (Diaz, 1995; Luscher *et al*., 1998; Wilson & Hartnett, 1998; Johnson *et al*., 2003a). Taxa of AM fungi are also known to differ in their responses to CO_2 enrichment (Klironomos *et al*., 1998; Treseder *et al*., 2003; Wolf *et al*., 2003).

Experimental assessment of mycorrhizal responses to CO_2 enrichment has been examined in very few plant–fungus combinations; and in most cases has combined genotypes of plants and AM fungi that do not co-occur in natural systems. The applicability of such studies may be limited because ecotypes of plants and AM fungi are likely to become coadapted to each other and to local edaphic conditions (Bever *et al*., 2001; Schultz *et al*., 2001; Sanders, 2002).

To understand the variation in AM function associated with different plant and fungal taxa and resource availability, we conducted three experiments that compared the impacts of CO_2 enrichment on the mycorrhizal function of many different plant–fungus combinations grown under high and low nutrient availability. The purpose of this research was to test the following hypotheses:

1. Enrichment of CO_2 should increase mycorrhizal benefits for plant growth.
2. Enrichment of soil nutrients should increase plant growth responses to CO_2 enrichment and decrease plant growth responses to AM fungi.

This individual-scale investigation focuses on plant biomass responses to AM symbioses. Other important benefits of AM associations such as interactions with root pathogens (Newsham *et al*., 1995) and soil structure (Miller & Jastrow, 2000) cannot be effectively examined in this series of experiments. However, these studies were designed to complement ecosystem-scale and community-scale experiments that do incorporate these higher-order AM functions. The ecosystem-scale experiment is a free-air CO_2 enrichment (FACE) experiment at Cedar Creek Minnesota, USA (Reich *et al*., 2001) in which AM fungi were studied (Wolf *et al*., 2003), but not manipulated. The community-scale experiment is a mesocosm study that examined AM effects on plant community responses to CO_2 enrichment (Johnson *et al*., 2003a).

Materials and methods

Experimental designs

Three separate experiments were conducted in 12 clear walled chambers (2.5 m × 1.3 m × 1.5 m, w × l × h) within a greenhouse at Northern Arizona University, Flagstaff Arizona, USA. Six chambers had ambient and six had elevated levels of atmospheric CO_2 (set points of 465 and 720 ppm, respectively, daylight hours only). At Flagstaff's atmospheric pressure (ca. 79 kPa), these concentrations provide CO_2 partial pressures of 36.7 and 56.9 Pa, equivalent to sea level concentrations of 368 and 562 ppm, respectively. Soil used in these experiments was collected adjacent to the FACE experiment at Cedar Creek, Minnesota and transported to Flagstaff, Arizona. The sandy soil is a Typic Udipsamment (Grigal *et al*., 1974) with approximately 43 $\mu g\, g^{-1}$ available P (Bray-1), and 95 $\mu g\, g^{-1}$ available N. For all three experiments, 25 cm deep × 6.4 cm diameter Deepots™ (Stuewe and Sons, Corvallis, OR, USA) were filled with 656 ml of soil (Experiments 2 and 3) or a soil–sand mixture (Experiment 1) that had been pasteurized by heating to 100 °C for 8 h on 2 consecutive days.

The three experiments differ in the number of plant species studied, species composition of the AM fungal inoculum, soil media, and nutrient treatments (Table 1). All treatments were replicated six times. Experiment 1 examined 14 plant species $\times 2$ CO_2 levels (aCO_2 and eCO_2) $\times 2$ AM treatments ($+$AM and $-$AM) $\times 6$ replicates, for a total of 366 plants. Experiment 2 examined 4 plant species $\times 2$ CO_2 levels (aCO_2 and eCO_2) $\times 3$ AM treatments (*Glomus* spp., Gigasporaceae spp., and $-$AM) $\times 2$ N levels ($+$N and $-$N) $\times 2$ P levels ($+$P and $-$P) $\times 6$ replicates, for a total of 576 plants. Experiment 3 examined 4 plant species $\times 2$ CO_2 levels (aCO_2 and eCO_2) $\times 3$ AM treatments (*Glomus intraradices*, *Gigaspora gigantea* and $-$AM) $\times 6$ replicates, for a total of 144 plants.

Plants and AM fungi

The 14 plant species examined in Experiment 1 included representatives of five functional groups: C_4 grasses, C_3 grasses, composites, legumes, and putative nonmycorrhizal forbs (Table 2). These species co-occur at Cedar Creek and are common in mesic grasslands in North America. Experiments 2 and 3 examined only four plant species: *Achillea millefolium*, *Koeleria cristata*, *Lespedeza capitata*, and *Schizachyrium scoparium*. Except for *Salsola kali*, all of the seeds used in this experiment were acquired from the same seed sources used by Reich *et al*., 2001 at the Cedar Creek FACE site. *Salsola* seeds were collected from a roadside near Flagstaff, AZ.

Cultures of AM fungi were established on leeks and celery from fresh Cedar Creek soil following the methods of Morton *et al*. (1993). The inoculum used in Experiment 1 was a mixture of many AM fungal species isolated from Cedar Creek (Table 1). Experiment 2 used two different fungal genera: a mixture of Cedar Creek *Glomus* species and a mixture of Cedar Creek Gigasporacea (i.e. *Gigaspora* and *Scutellospora* spp.). Experiment 3 used pure cultures of *Glomus intraradices* and *Gigaspora gigantea* kindly provided by John Klironomos at the University of Guelph, Canada.

Glomus inoculum consisted of colonized roots and spores and Gigasporaceae inoculum consisted of spores. In Experiment 1, a band of 5.5 g of fresh root fragments and AM spores was layered 6 cm from the top of each Deepot™ and covered with 3 cm of pasteurized soil. The $-$AM treatments were established in the same way except 5.5 g of autoclaved root fragments and AM spores were used. Communities of microorganisms (other than AM fungi) were equalized by adding a microbial wash to the $-$AM treatments. The microbial wash was prepared from water that had been used to extract spores and root fragments from the leek and celery cultures. This water was filtered through a 25 μm sieve four times, and 5 mL was applied to the $-$AM treatments. Inoculum and microbial wash were applied similarly in the two other experiments except that 32 g of colonized roots, soil, and spores were used in Experiment 2 and 4.7 g of perlite + colonized roots and spores were used in Experiment 3.

Growth conditions

Air temperature monitored at 10 min intervals within each of the 12 greenhouse chambers varied diurnally and among experiments with average daily minima and maxima (°C) of 17.1 and 32.2 in Experiment 1, 16.9 and 28.5 in Experiment 2, and 18.1 and 25.8 in Experiment 3 (Table 1). Maximum daytime PAR irradiance was about 1200 $\mu mol\, m^{-2}\, s^{-1}$ at noon on clear days near the summer solstice, and as low as 400 $\mu mol\, m^{-2}\, s^{-1}$ on clear days during winter. Plants were watered as needed with water that had been passed through a charcoal filter. Once each week, all of the plants in Experiment 1 were watered using a nutrient solution that was modified from Sylvia & Hubbell's (1986) formulation for aeroponic culture of mycorrhizae, and contained: 433 $mg\, L^{-1}$ KNO_3, 8.4 $mg\, L^{-1}$ $Ca(NO_3)_2 \cdot 4H_2O$, 199 $mg\, L^{-1}$ $CaSO_4 \cdot 2H_2O$, 130 $mg\, L^{-1}$ K_2SO_4, 72 $mg\, L^{-1}$ $MgSO_4$, 0.86 $mg\, L^{-1}$ H_3BO_4, 0.54 $mg\, L^{-1}$ $MnCl \cdot 4H_2O$, 0.07 $mg\, L^{-1}$ $ZnSO_4 \cdot 7H_2O$, 0.03 $mg\, L^{-1}$ NaCl, and 0.02 $mg\, L^{-1}$ $CuSO_4 \cdot 5H_2O$. In Experiment 2, twice each week the $+$N plants were watered with a solution containing 433 $mg\, L^{-1}$ KNO_3, and $+$P plants received a solution containing 44 $mg\, L^{-1}$ KH_2PO_4. Plants in Experiment 3 received only water and no supplemental nutrients. Plants were harvested before they became root-bound. In Experiment 1, fast growing *Echinochloa crusgalli* was harvested at 8 weeks, the other plant species were harvested between 10.5 and 16 weeks. In Experiments 2 and 3, *Schizachyrium scoparium* was harvested at 10.5 weeks, *Achillea millefolium* was harvested at 14 weeks, and *Koeleria cristata*, and *Lespedeza capitata* were harvested at 16 weeks.

Response variables

At harvest, plants were carefully removed from the soil and their roots were gently washed. Shoots were cut from roots, dried, and weighed. Roots were divided into two sub-samples and the fresh weight was determined for both. One subsample was stained using the technique of Koske & Gemma (1989), and examined for percent of root length colonized by AM fungi using the method of McGonigle *et al*. (1990). The other subsample was dried, and its fresh/dry mass ratio was used to calculated total root dry mass. Mycorrhizal

Table 1 Environmental conditions and materials used in Experiments 1, 2, and 3

Experiment dates, chamber temperatures*	Plants	AM fungal inoculum	Soil media	Nutrients
Experiment 1 June 2001 through August/ October 2001 Mean °C: 23.2 ± 1.0 Min °C: 17.1 ± 1.9 Max °C: 32.2 ± 1.9	14 species, 366 plants	One mixture of Cedar Creek *Glomus* and *Acaulospora* spp: *G. aggregatum/intraradices, G. clarum, G. constrictum, A. scrobiculata, A. spinosa,* and *A. trappei*	One part Cedar Creek soil + three parts silica sand	All treatments received Sylvia and Hubbell's nutrient solution weekly
Experiment 2 August 2002 through December 2002 Mean °C: 21.4 ± 2.4 Min. °C: 16.9 ± 2.0 Max. °C: 28.5 ± 2.9	Four species, 576 plants	Two mixtures of Cedar Creek spp: *Glomus* mixture: *Glomus aggregatum/intraradices, G. clarum, G. claroidium, G. fasciculatum* Gigasporaceae mixture: *Gigaspora gigantea, Gi. margarita, Scutellospora calospora, Scutellospora* sp. (amber)	100% Cedar Creek soil	Factorial N and P treatments received N and P twice a week
Experiment 3 November 2002 through February 2003 Mean °C: 20.4 ± 1.6 Min. 18.1 ± 1.3 Max. 25.8 ± 3.1	Four species, 144 plants	Two pure-cultures of non-endemic spp: *Glomus intraradices* and *Gigaspora gigantea*	100% Cedar Creek soil	No nutrients, only filtered water

*Average daily mean, maximum and minimum (± SD) over entire experiment for all 12 chambers.
AM, arbuscular mycorrhizal.

Table 2 Results from Experiment 1

Plant species	Species abbreviation	*F*-ratios		
		CO_2	AM	$CO_2 \times$ AM
C_4 grasses				
Andropogon gerardi Vitman	ANGE	ns	ns	4.25^{ψ}
Bouteloua gracilis(Willd. ex Kunth) Lag. ex Griffiths	BOGR	↓ 4.92*	ns	ns
Echinochloa crus-galli L. (Beauv.)	ECCR	ns	ns	ns
Schizachyrium scoparium(Michauux) Nash	SCSC	ns	↑ 8.65**	ns
C_3 grasses				
Koeleria cristata Pers	KOCR	↑ 8.08*	$\downarrow 4.12^{\psi}$	ns
Poa pratensis L	POPR	↑ 17.68***	ns	10.94**
Composites				
Achillea millefolium L	ACMI	↑ 5.37*	ns	ns
Heliopsis helianthoides L.	HEHE	ns	ns	ns
Solidago rigida L	SORI	ns	ns	ns
Legumes				
Lespedeza capitata (Michaux)	LECA	ns	↑ 17.80**	ns
Lupinus perennis L†	LUPE	ns	ns	ns
Petalostemum velosum Nutt.	PEVE	ns	ns	ns
Non-mycorrhizal forbs				
Berteroa incana L.	BEIN	ns	↓ 5.04*	9.14**
Salsola kali L.	SAKA	ns	ns	ns

Effect of CO_2 enrichment and AM fungi on total plant biomass (TDW) as indicated by the *F*-ratios of two-way ANOVA. Arrows indicate factors that significantly increased ↑ or decreased ↓ the TDW of particular plant species. Significant $CO_2 \times$ AM interactions are described in the text, ns indicates *F*-ratios were not significant.
$^{\psi}$*F*-ratios were significant at $P \leq 0.10$; **F*-ratios were significant at $P \leq 0.05$; ***F*-ratios were significant at $P \leq 0.01$; ****F*-ratios were significant at $P \leq 0.001$; †*Lupinus* is both a legume and a nonmycorrhizal forb.
AM, arbuscular mycorrhizal; TDW, total dry mass.

responsiveness (MR) was calculated by comparing the total dry mass (TDW) of each species with and without mycorrhizae: MR = ln(TDW + AM plant/TDW−AM plant). For these calculations, + AM and −AM plants were paired so that they shared the same greenhouse chamber, CO_2, and nutrient treatments.

Total Kjeldahl N and P in dry shoots of *Achillea*, *Koeleria*, and *Schizachyrium* from Experiments 2 and 3 were determined colorimetrically by flow injection analysis using a Lachat Automated Ion Analyzer. Aboveground tissue samples were dried in a 60 °C oven for 72 h and then ground on a Wiley Mill to pass through a 20 μm mesh sieve. Samples, weighing 0.05 g, were digested on a block digester for 5 h at 350 °C. Digested samples were refrigerated until 1 day prior to analysis. Tissue N and P were not determined for *Lespedeza* or for plants fertilized with N and P (in Experiment 2).

Statistical analyses

All response variables were analyzed using ANOVA with the full model of all experimental treatments. This allowed us to test the main effects and two-way interactions of: CO_2 level, N and P level (Experiment 2), mycorrhizal status, and the random blocking effect of chamber nested within CO_2 level (no interactions tested). Root colonization data were arc-sine square root transformed prior to ANOVA. All statistical analyses were performed using JMP 4.0 (SAS, 1997).

Results

Root colonization

Roots of plants inoculated with AM fungi became colonized and roots of −AM treatments remained uncolonized except for a few individuals which had very low levels of colonization (0.06–0.7%). Mycorrhizal inoculation of the nonmycorrhizal species in Experiment 1 did not generate colonization (*Salsola* and *Berteroa*) or only very low levels of colonization (<1% in *Lupinus*). Levels of AM colonization varied greatly among plant and fungal taxa and experiments. There were no consistent patterns in the responses of AM colonization to CO_2 and nutrient treatments. Root colonization increased in response to CO_2 enrichment in *Poa* (Experiment 1, $F = 6.55$, $P = 0.03$) and *Lespedeza*

(Experiment 2, $F = 6.95$, $P = 0.06$); but it decreased in response to CO_2 enrichment in *Koeleria* (Experiment 1, $F = 5.35$, $P = 0.04$) and *Schizachyrium* (Experiment 2, $F = 6.32$, $P = 0.02$; Experiment 3, $F = 5.21$, $P = 0.02$). Nitrogen and phosphorus treatments did not significantly affect AM colonization with the exception that nitrogen enrichment increased colonization in *Koeleria* in Experiment 2 ($F = 5.03$, $P = 0.03$).

Experiment 1

The 14 plant species varied in their responses to CO_2 enrichment and AM fungi (Table 2). Elevated CO_2 significantly increased the TDW of both C_3 grasses (*Koeleria* and *Poa*) and one of the composites (*Achillea*). In contrast, TDW of one of the C_4 grasses (*Bouteloua*) was significantly reduced by CO_2 enrichment. Inoculation with AM fungi increased the TDW of one C_4 grass (*Schizachyrium*) and one legume (*Lespedeza*), and it decreased the TDW of *Koeleria* and the nonmycorrhizal forb *Berteroa*. Furthermore, there was a significant $CO_2 \times$ AM interaction in the growth responses of *Berteroa*, *Poa*, and the C_4 grass *Andropogon* (Table 2). MR was not significantly influenced by CO_2 enrichment in 11 of the 14 species; however, it caused MR to become more negative in *Berteroa* and *Poa* and it increased MR of *Petalostemum* from negative to neutral (Fig. 1).

Experiment 2

Total biomass of three of the four plant species studied in Experiment 2 responded positively to CO_2 enrichment (Table 3). None of the plant species responded directly to AM fungi, although there was a significant $CO_2 \times$ AM interaction for TDW of *Lespedeza* and *Schizachyrium*. For both species, plants colonized with Gigasporaceae were larger at elevated than at ambient CO_2, while those colonized with *Glomus* were larger at ambient than at elevated CO_2. Nitrogen enrichment strongly increased TDW of *Koeleria* and it decreased TDW of *Schizachyrium* and *Lespedeza*. Also, there was a significant N × AM effect on TDW of *Koeleria*; at low N, −AM plants were significantly larger than those colonized by Gigasporaceae. None of the plants in Experiment 2 responded directly to P enrichment, however there was a significant $CO_2 \times$ P interaction on TDW of *Schizachyrium*. At high P, *Schizachyrium* grown at ambient CO_2 were significantly larger than those grown at elevated CO_2. Also, there was a significant N × P interaction on TDW of *Koeleria*; among plants grown at low N, those given high P were significantly smaller than those given low P (Table 3).

MR of the four species was influenced differently by the experimental treatments. Enrichment of P decreased the MR of *Achillea* ($F = 3.9$, $P = 0.05$, data not shown). In contrast, enrichment of N increased MR of *Achillea*, *Koeleria* and *Lespedeza* (Fig. 2). Also, a $CO_2 \times$ N interaction influenced the MR of *Achillea* and *Koeleria*. In both species, N enrichment caused MR to increase in elevated but not ambient CO_2. The composition of the AM fungal inoculum interacted with CO_2 in *Lespedeza* and *Schizachyrium*. In both species, CO_2 enrichment increased the MR of plants inoculated with Gigasporaceae and decreased the MR of plants inoculated with *Glomus* (Fig. 2).

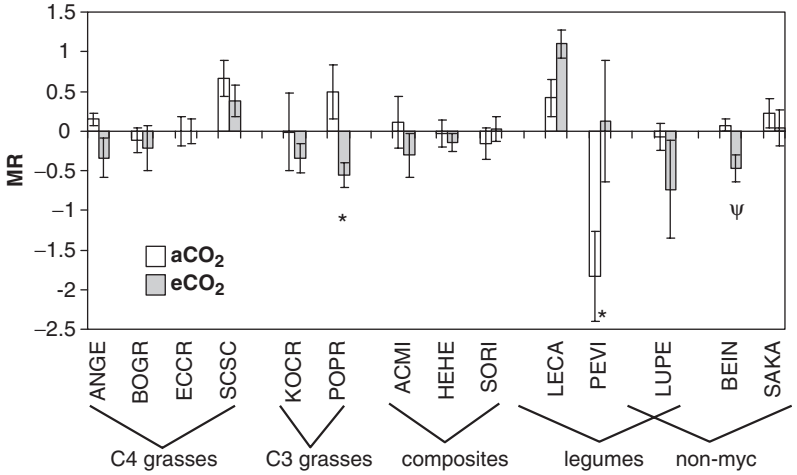

Fig. 1 The effects of CO_2 enrichment on mycorrhizal responsiveness total dry mass (TDW); arbuscular mycorrhizal (AM) (MR, = ln (TDW + AM plant/TDW−AM plant)) of the 14 plant species in Experiment 1. Species abbreviations are defined in Table 2. Bars represent mean values with standard error lines ($N = 6$), unshaded bars represent MR at ambient CO_2 and shaded bars represent MR at elevated CO_2. Positive values indicate AM mutualism and negative values indicate AM parasitism. $^{\psi}$, * indicate that within a species, mean MR in ambient and elevated CO_2 are significantly different at $P \leq 0.10$ and ≤ 0.05 respectively.

Table 3 Results from Experiment 2

	F-ratios for main effects				*F*-ratios for interactions†			
Plant species	CO_2	AM	N	P	$CO_2 \times$ AM	$CO_2 \times$ P	N × AM	N × P
Achillea millefolium	↑ 4.44$^{\psi}$	ns	ns	ns	ns	ns	ns	ns
Koeleria cristata	↑ 14.95**	ns	↑ 77.35***	ns	ns	ns	3.80*	5.88**
Lespedeza capitata	↑ 5.26*	ns	↓ 3.15$^{\psi}$	ns	9.22***	ns	ns	ns
Schizachyrium scoparium	ns	ns	↓ 4.31*	ns	3.65*	5.02*	ns	ns

Effect of CO_2 enrichment, AM fungi, N, and P enrichment on total plant biomass (TDW) as indicated by the *F*-ratios of two-way ANOVA. Arrows indicate factors that significantly increased ↑ or decreased ↓ the TDW of particular plant species. Significant interactions are described in the text, ns indicates *F*-ratios were not significant.
$^{\psi}$*F*-ratios were significant at $P \leq 0.10$; *F-ratios were significant at $P \leq 0.05$; **F-ratios were significant at $P \leq 0.01$; ***F-ratios were significant at $P \leq 0.001$. †None of the $CO_2 \times$ N or P × AM interactions were significant.
AM, arbuscular mycorrhizal; TDW, total dry mass.

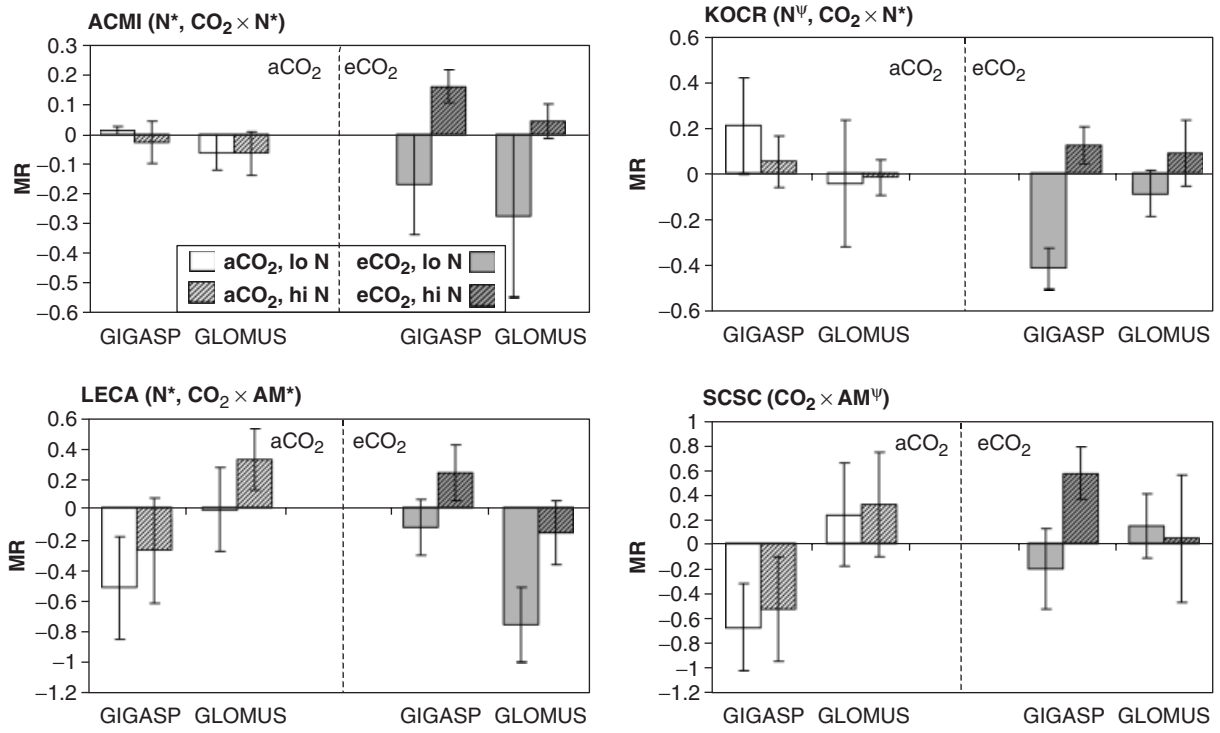

Fig. 2 The effects of CO_2 enrichment and arbuscular mycorrhizal (AM) taxa on mycorrhizal responsiveness (MR, = ln(TDW + AM plant/TDW−AM plant)) of the four plant species studied in Experiment 2. Bars represent mean values with standard error lines ($N = 6$). Unshaded bars represent MR at ambient CO_2 and shaded bars represent MR at elevated CO_2; striped bars represent high nitrogen treatments. Positive values indicate AM mutualism and negative values indicate AM parasitism. The significance levels of relevant treatments and interactions, as detected by ANOVA, are listed for each response, $^{\psi}$, and * indicate $P \leq 0.10$ and ≤ 0.05, respectively.

Experiment 3

Unlike the previous two experiments, none of the plant species had a significant biomass response to CO_2 enrichment in Experiment 3 (Table 4). Like the previous experiments, the TDW of *Schizachyrium* was influenced by AM fungi or a $CO_2 \times$ AM interaction. *Schizachyrium* grew significantly larger when inoculated with Gigasporaceae vs. *Glomus*, and CO_2 enrichment further increased the benefits of this fungus. MR of *Schizachyrium* was significantly greater at elevated CO_2 than ambient, and in plants inoculated with Gigasporaceae than those inoculated with *Glomus* (Figs 3 and 4). In contrast, MR of *Lespedeza* inoculated with *Glomus* was significantly lower at elevated CO_2 than at ambient CO_2 (Fig. 3).

Table 4 Results from Experiment 3

	F-ratios		
Plant species	CO_2	AM	$CO_2 \times$ AM
Achillea millefolium	ns	ns	ns
Koeleria cristata	ns	ns	ns
Lespedeza capitata	ns	ns	ns
Schizachyrium scoparium	ns	↑ 11.92***	3.62*

Effect of CO_2 enrichment and AM fungi on total plant biomass (TDW) as indicated by the *F*-ratios of two-way ANOVA. Arrows indicate factors that significantly increased ↑ or decreased ↓ TDW of particular plant species. Significant $CO_2 \times$ AM interactions are described in the text, ns indicates *F*-ratios were not significant.
*F-ratios were significant at $P \leq 0.05$; ***F-ratios were significant at $P \leq 0.001$.
AM, arbuscular mycorrhizal; TDW, total dry mass.

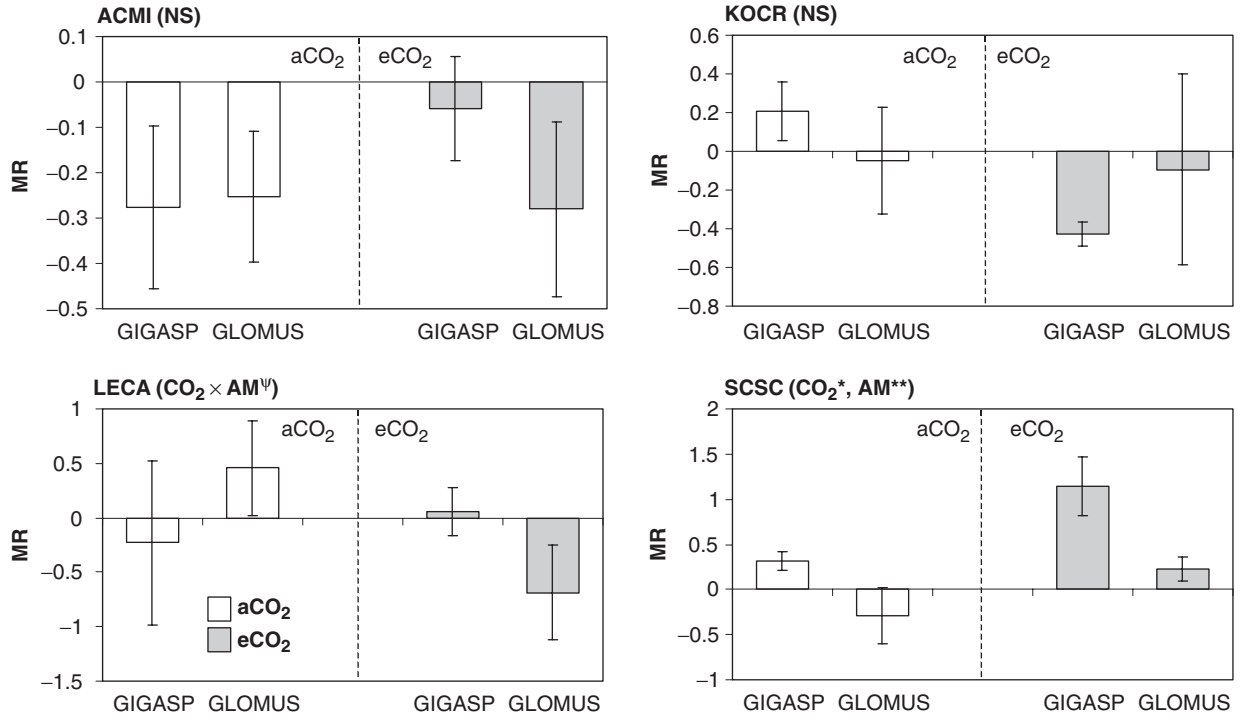

Fig. 3 The effects of CO_2 enrichment and arbuscular mycorrhizal (AM) taxa on mycorrhizal responsiveness (MR, = ln(TDW + AM plant/TDW−AM plant)) of the four plant species studied in Experiment 3. Bars represent mean values with standard error lines ($N = 6$). Unshaded bars represent MR at ambient CO_2 and shaded bars represent MR at elevated CO_2. Positive values indicate AM mutualism and negative values indicate AM parasitism. The significance levels of relevant treatments and interactions, as detected by ANOVA, are listed for each response, $^{\Psi}$, and ** indicate $P \leq 0.10$ and ≤ 0.01, respectively.

Plant tissue N and P

Across the three plant species that were analyzed, foliar %N and %P were significantly lower in plants from Experiment 2 than from Experiment 3 (for N: $F = 228.7$, $P < 0.0001$; for P: $F = 121.4$, $P < 0.0001$). Foliar %N was significantly lower in plants grown at elevated CO_2 ($F = 15.5$, $P = 0.05$), but CO_2 enrichment had no effect on foliar %P. There was no significant effect of AM inoculation on either %N or %P in any of the species in either experiment.

Discussion

Our results indicate that enrichment of CO_2 does not uniformly increase mycorrhizal benefits for plant growth. To the contrary, there is variability in responses and when the AM fungal community is dominated by

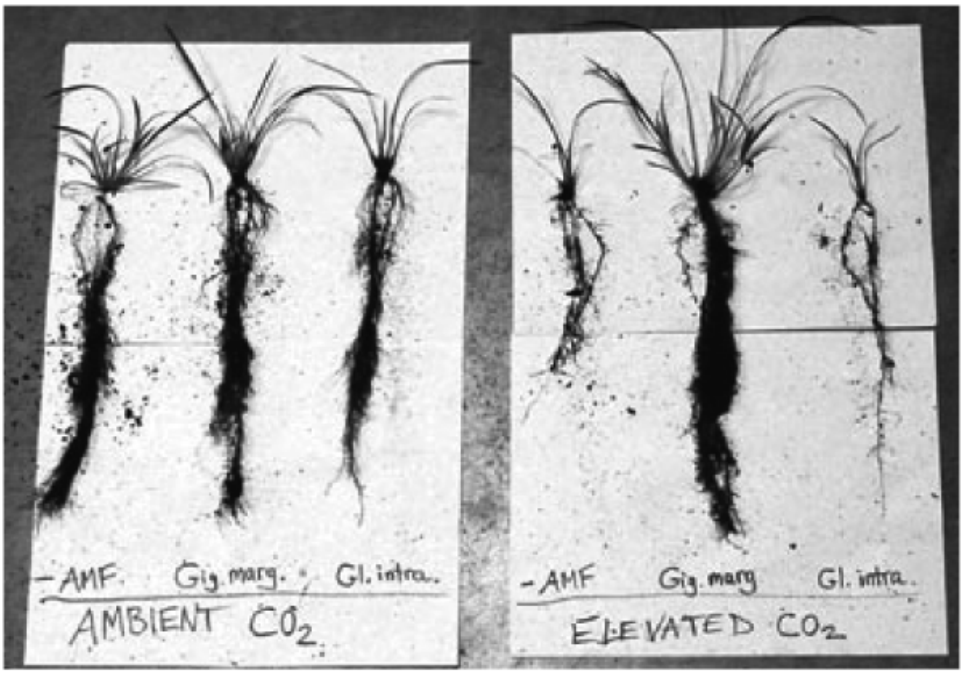

Fig. 4 Photograph of *Schizachyrium* that has been grown at ambient (left) or elevated (right) CO_2 in the absence of arbuscular mycorrhizal (AM) fungi (−AMF), with *Gigaspora gigantea* (Gig marg.) or with *Glomus intraradices* (Gl. intra.) in Experiment 3.

Glomus species, CO_2 enrichment may actually reduce the beneficial effects of mycorrhizae on plant biomass (e.g. in *Lespedeza*). Mechanisms for this result are unknown; however studies show that members of Glomaceae and Gigasporaceae differ in their mutualistic exchange of carbon and nutrients (Douds & Schenck, 1990; Pearson & Jakobsen, 1993; Smith *et al.*, 2004). It is possible that differences in the C sink-strength among AM fungal taxa influence their responses to changes in host plant physiology that accompany CO_2 enrichment. Future studies are necessary to assess this possibility.

It is well known that root colonization levels are often not related to mycorrhizal function (McGonigle, 1988; Smith *et al.*, 2004). Consequently, in our experiments, the absence of a consistent response of AM colonization to CO_2 or nutrient enrichment despite significant functional responses is not unexpected, and it further underscores the importance of measuring AM variables other than root colonization. Quantification of functional changes in mycorrhizal associations can only be measured using a functional parameter such as MR, not through measuring a structural parameter such as root colonization.

Our hypothesis, that enrichment of soil nutrients should increase plant growth responses to CO_2 enrichment and decrease plant growth responses to AM fungi, was also not supported by our data. In Experiment 2, none of the plant species exhibited a significant TDW response to the $N \times CO_2$ interaction and only *Schizachyrium* showed a significant $P \times CO_2$ interaction. However, the outcome of this interaction was opposite to that predicted by the hypothesis: P enrichment increased the TDW of plants grown at ambient CO_2 significantly more than those grown at elevated CO_2. As expected, P enrichment reduced the MR of *Achillea*, but contrary to expectations, enrichment of N increased rather than decreased MR of three of the four species studied in Experiment 2. Also, there were significant $N \times CO_2$ interactions on MR of *Achillea* and *Koeleria*. In both species, N enrichment increased MR at elevated but not ambient CO_2. These findings suggest that plants and AM fungi may be competing for N when C is not in limiting supply. This idea is supported by the fact that the Cedar Creek soil used in this experiment has very low levels of N (Johnson *et al.*, 2003b). Cedar Creek soil also has very high levels of P, and that may help account for the unexpected observation that AM colonization did not increase the percent tissue P in Experiments 2 and 3.

We observed considerable interexperiment variability in the responses of individual plant species. Differences between the three experiments could result from differences in fungal inoculum or environmental conditions. Different AM fungi were used in each experiment, ranging from a diverse community of many species in Experiment 1 to genus-specific consortia in Experiment 2 to single isolates of *Glomus intraradices* and *Gigaspora gigantea* in Experiment 3. Given these

differences, we find it striking that in both Experiments 2 and 3 the MR of *Schizachyrium* inoculated with Gigasporaceae increased in response to CO_2 enrichment and the MR of *Lespedeza* inoculated with Glomaceae decreased in response to CO_2 enrichment. This finding suggests that Glomaceae and Gigasporaceae may have fundamentally different carbon exchange relationships with their host plants.

Different temperature and light conditions among the three experiments could also explain some of the interexperiment variability. Both of these environmental factors have been shown to influence mycorrhizal development in field experiments (Heinemeyer *et al*., 2003; Staddon *et al*., 2003, 2004). Furthermore, Gavito *et al*. (2003) showed that temperature influences AM function more strongly than CO_2 enrichment. It is not possible to determine whether our interexperiment differences were generated by differences in the species composition of the AM fungal inocula, differences in the greenhouse conditions, or interactions among these biotic and abiotic factors. However, we can safely conclude that when making predictions about mycorrhizal function, it is unwise to extrapolate findings from experiments that are conducted using a narrow range of plant hosts, AM fungi, and environmental conditions.

Plant responses observed in these three individual-scale experiments corroborate those observed in our community-scale companion study (Johnson *et al*., 2003a), in which all 14 species were grown together in experimental mesocosms at elevated and ambient levels of CO_2 and soil nitrogen. In both studies, plant species varied considerably in their responses to atmospheric CO_2 levels and the presence or absence of AM fungi. Elevated CO_2 often enhanced growth of C_3 species (e.g. *Koeleria*, *Poa*, and *Achillea*) but not C_4 species (e.g. *Bouteloua* and *Schizachyrium*), and plants with coarse roots such as *Lespedeza* benefited greatly from AM fungi, while plants with fine root systems such as *Koeleria* and *Berteroa* often grew more slowly in the presence of AM fungi. Our ecosystem-scale companion study (Wolf *et al*., 2003) showed that AM fungal species differ in their responses to CO_2 enrichment and plant diversity. In plant monocultures, spore densities of *Glomus clarum* increased while densities of *G. fasciculatum* decreased in response to CO_2 enrichment but these responses disappeared in polycultures composed of 16 plant species. These findings, combined with those of the present study, indicate that species of plants and AM fungi are individualistic in their responses to CO_2 enrichment, and the functioning of pairs of plants and AM fungi also respond differently to CO_2 enrichment. Furthermore, some patterns are sensitive to the scale of the experimental system (i.e. individual, community, or ecosystem) while other patterns are not.

Future studies should be designed to dove-tail multiple experimental scales and account for biotic and abiotic mediators of mycorrhizal function. Efforts should be made to grow naturally co-occurring plant and AM fungal genotypes in their native soil under realistic temperature and light regimes because mycorrhizal function is controlled by interactions among plants, mycorrhizal fungi, and environmental factors, particularly factors related to resource availability (Johnson *et al*., 1997). This complexity must be considered when making predictions of community responses to atmospheric CO_2 enrichment.

Acknowledgements

This work was funded by a National Science Foundation grant to Nancy Johnson and George Koch (DEB:9806529). Melissa Reyes was supported through a Hooper Undergraduate Research grant and the Undergraduate Mentoring in Environmental Biology Program at NAU (DEB-0080156). Ashley Panter was supported by a NASA Space Grant Internship. We appreciate helpful suggestions by Pål Axel Olsson and an anonymous reviewer on an earlier draft of this manuscript.

References

Bever JD, Schultz PA, Pringle A *et al*. (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *BioScience*, **51**, 923–931.

Diaz S (1995) Elevated CO_2 responsiveness, interactions at the community level and plant functional types. *Journal of Biogeography*, **22**, 289–295.

Douds DD, Schenck NC (1990) Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. *New Phytologist*, **116**, 621–627.

Fitter AH (1991) Costs and benefits of mycorrhizas: implications for functioning under natural conditions. *Experientia*, **47**, 350–355.

Fitter AH, Heinemeyer A, Staddon PL (2000) The impact of elevated CO_2 and global climate change on arbuscular mycorrhizas: a mycocentric approach. *New Phytologist*, **147**, 179–187.

Gavito ME, Schweiger P, Jakobsen I (2003) P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO_2 enrichment. *Global Change Biology*, **9**, 106–116.

Graham JH, Eissenstat DM (1994) Host genotype and the formation and function of VA mycorrhizae. *Plant and Soil*, **159**, 179–185.

Grigal DF, Chamberlain LM, Finney HR, Wroblewski DV *et al*. (1974) *Soils of the Cedar Creek Natural History Area*. University of Minnesota Agricultural Experiment Station, Miscellaneous Report number 123, 47 pp.

Heinemeyer A, Ridgway KP, Edwards EJ *et al*. (2003) Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. *Global Change Biology*, **10**, 52–64.

Hoeksema JD, Bruna EM (2000) Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. *Oecologia*, **125**, 321–330.

Jifton JL, Graham JH, Drouillard DL *et al*. (2002) Growth depression of mycorrhizal *Citrus* seedlings grown at high phosphorus supply is mitigated by elevated CO_2. *New Phytologist*, **153**, 133–142.

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications*, **3**, 749–757.

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–585.

Johnson NC, Wolf J, Koch GW (2003a) Interactions among mycorrhizae, atmospheric CO_2 and soil N impact plant community composition. *Ecology Letters*, **6**, 532–540.

Johnson NC, Rowland DL, Corkidi L *et al*. (2003b) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology*, **84**, 1895–1908.

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, **84**, 2292–2301.

Klironomos J, Ursic M, Rillig M *et al*. (1998) Inter-specific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO_2. *New Phytologist*, **138**, 599–605.

Koch KE, Johnson CR (1984) Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and nonmycorrhizal root systems. *Plant Physiology*, **75**, 26–30.

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect mycorrhizas. *Mycological Research*, **92**, 486–488.

Lovelock C, Kyllo D, Popp M *et al*. (1997) Symbiotic vesicular-arbuscular mycorrhizae influence maximum rates of photosynthesis in tropical tree seedlings grown under elevated CO_2. *Australian Journal of Plant Physiology*, **24**, 185–194.

Luscher A, Hendrey GR, Nosberger J (1998) Long-term responsiveness to free air CO_2 enrichment of functional types, species and genotypes of plants from fertile permanent grasslands. *Oceologia*, **113**, 37–45.

McGonigle TP (1988) A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. *Functional Ecology*, **2**, 473–478.

McGonigle TP, Miller MH, Evans DG *et al*. (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Miller RM, Jastrow JD (2000) Mycorrhizal fungi influence soil structure. In: *Arbuscular Mycorrhizas: Physiology and Function* (eds Kapulnik Y, Douds Jr. DD Jr.), pp. 3–18. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Miller RM, Miller SP, Jastrow JD *et al*. (2002) Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. *New Phytologist*, **155**, 149–162.

Morton JB, Bentivenga SP, Wheeler WW (1993) Germ plasm in the international collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon*, **48**, 491–528.

Newsham K, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, **10**, 407–411.

O'Neill E (1994) Responses of soil biota to elevated atmospheric carbon dioxide. *Plant and Soil*, **165**, 55–65.

Pearson JN, Jakobsen I (1993) Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. *New Phytologist*, **124**, 481–488.

Reich PB, Knops J, Tilman D *et al*. (2001) Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. *Nature*, **410**, 809–812.

Rillig MC (2004) Arbuscular mycorrhize and terrestrial ecosystem processes. *Ecology Letters*, **7**, 740–754.

Sanders IR (2002) Ecology and evolution of multigenomic arbuscular mycorrhizal fungi. *American Naturalist*, **160** (Suppl.), s128–s141.

SAS (1997) *JMP Statistical Discovery Software, Version 4*. SAS Institute Inc., Cary, NC, USA.

Schultz PA, Miller RM, Jastrow JD *et al*. (2001) Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. *American Journal of Botany*, **88**, 1650–1656.

Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlation with mycorrhizal reponses in growth or total P uptake. *New Phytologist*, **162**, 511–524.

Staddon PL, Gregersen R, Jakobsen I (2004) The response of two *Glomus* mycorrhizal fungi and a fine endophyte to elevated atmospheric CO_2, soil warming and drought. *Global Change Biology*, **10**, 1909–1921.

Staddon PL, Thompson K, Jakobsen I *et al*. (2003) Mycorrhizal fungal abundance is affected by long-term climatic manipulations in the field. *Global Change Biology*, **9**, 186–194.

Sterner RW, Elser JJ (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ, USA.

Sylvia DM, Hubbell DH (1986) Growth and sporulation of vesicular-arbuscular mycorrhizal fungi in aeroponic and membrane systems. *Symbiosis*, **1**, 259–268.

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, **164**, 347–355.

Treseder KK, Egerton-Warburton LM, Allen MF *et al*. (2003) Alteration of soil carbon pools and communities of mycorrhizal fungi in chaparral exposed to elevated CO_2. *Ecosystems*, **6**, 786–796.

Wilson G, Hartnett D (1998) Interspecific variation in plant responses to mycorrhizal colonization in tall grass prairie. *American Journal of Botany*, **85**, 1732–1738.

Wolf J, Johnson NC, Rowland DL *et al*. (2003) Elevated carbon dioxide and plant species richness impact arbuscular mycorrhizal fungal spore communities. *New Phytologist*, **157**, 579–588.

Wright DP, Scholes JD, Read DJ (1998a) Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant, Cell and Environment*, **21**, 209–216.

Wright DP, Read DJ, Scholes JD (1998b) Mycorrhizal sink strength influences whole plant carbon balance of *Trifofium repens* L. *Plant, Cell and Environment*, **21**, 881–891.